

What is claimed is:

Sub A27
1. A method for identifying a modulator of quorum sensing signaling in bacteria, said method comprising:

providing a cell which comprises a quorum sensing controlled gene, wherein said cell is responsive to a quorum sensing signal molecule such that a detectable signal is generated;

10 contacting said cell with a quorum sensing signal molecule in the presence and absence of a test compound;

and detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria.

15 2. The method of claim 1, wherein said cell further comprises means for generating said detectable signal.

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20 3. The method of claim 2, wherein said signal generation means comprises a reporter gene, and wherein said quorum sensing signal molecule causes transcription of said reporter gene, said transcription providing said detectable signal.

4. The method of claim 3, wherein said reporter gene is operatively linked to a regulatory sequence of said quorum sensing controlled gene.

25 5. The method of claim 4, wherein said reporter gene is selected from the group consisting of *ADE1*, *ADE2*, *ADE3*, *ADE4*, *ADE5*, *ADE7*, *ADE8*, *ASP3*, *ARG1*, *ARG3*, *ARG4*, *ARG5*, *ARG6*, *ARG8*, *ARO2*, *ARO7*, *BARI*, *CAT*, *CHO1*, *CYS3*, *GAL1*, *GAL7*, *GAL10*, *GFP*, *HIS1*, *HIS3*, *HIS4*, *HIS5*, *HOM3*, *HOM6*, *ILV1*, *ILV2*, *ILV5*, *INO1*,
30 *INO2*, *INO4*, *lacZ*, *LEU1*, *LEU2*, *LEU4*, *luciferase*, *LYS2*, *MAL*, *MEL*, *MET2*, *MET3*, *MET4*, *MET8*, *MET9*, *MET14*, *MET16*, *MET19*, *OLE1*, *PHO5*, *PRO1*, *PRO3*, *THR1*, *THR4*, *TRP1*, *TRP2*, *TRP3*, *TRP4*, *TRP5*, *URA1*, *URA2*, *URA3*, *URA4*, *URA5* and *URA10*.

35 6. The method of claim 5, wherein said reporter gene is *lacZ* or *GFP*.

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7. The method of claim 1, wherein said cell does not express said quorum sensing signal molecule.

8. The method of claim 7, wherein said quorum sensing signal molecule is produced by a second cell.

9. ~~The method of claim 1, wherein said cell is a prokaryote or eukaryote.~~

10. The method of claim 9, wherein said cell is a bacterium.

11. The method of claim 8, wherein said second cell is a prokaryote or
10 eukaryote.

12. The method of claim 11, wherein said second cell is a bacterium.

13. The method of claim 10 or 12, wherein said bacterium is a gram negative bacterium.

14. The method of claim 13, wherein said gram negative bacterium is *Pseudomonas aeruginosa*.

15. The method of claim 10, wherein said bacterium is a mutant strain of *Pseudomonas aeruginosa* which comprises a regulatory sequence of a quorum sensing controlled gene operatively linked to a reporter gene, wherein in said mutant strain, *lasI* and *rhlI* are inactivated.

16. The method of claim 12, wherein said second cell is wild type
25 *Pseudomonas aeruginosa*.

~~17. The method of claim 1, wherein said quorum sensing controlled gene is endogenous to said cell.~~

30 18. The method of claim 10, wherein said quorum sensing controlled gene
encodes a virulence factor.

19. The method of claim 10, wherein said quorum sensing controlled gene encodes a polypeptide which inhibits a bacterial host defense mechanism.

20. The method of claim 10, wherein said quorum sensing controlled gene encodes a polypeptide which regulates biofilm formation.

Sub 32

5 lactone.

lactone analog.

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Sub
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sensing signal molecule.

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quorum sensing signal molecule;

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compound as a modulator of quorum sensing signaling in *Pseudomonas aeruginosa*.

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inactivated.

Parameter	Unit	Value	Standard Error	t-Statistic	p-Value
Intercept		1.0000	0.0000	1.0000	0.0000
Age	Years	0.0000	0.0000	0.0000	0.0000
Age squared	Years squared	0.0000	0.0000	0.0000	0.0000
Age cubed	Years cubed	0.0000	0.0000	0.0000	0.0000
Age quart	Years quart	0.0000	0.0000	0.0000	0.0000
Age quint	Years quint	0.0000	0.0000	0.0000	0.0000
Age sext	Years sext	0.0000	0.0000	0.0000	0.0000
Age sept	Years sept	0.0000	0.0000	0.0000	0.0000
Age oct	Years oct	0.0000	0.0000	0.0000	0.0000
Age non	Years non	0.0000	0.0000	0.0000	0.0000
Age dec	Years dec	0.0000	0.0000	0.0000	0.0000
Age ele	Years ele	0.0000	0.0000	0.0000	0.0000
Age twel	Years twel	0.0000	0.0000	0.0000	0.0000
Age thir	Years thir	0.0000	0.0000	0.0000	0.0000
Age four	Years four	0.0000	0.0000	0.0000	0.0000
Age fiv	Years fiv	0.0000	0.0000	0.0000	0.0000
Age six	Years six	0.0000	0.0000	0.0000	0.0000
Age sev	Years sev	0.0000	0.0000	0.0000	0.0000
Age eigh	Years eigh	0.0000	0.0000	0.0000	0.0000
Age nine	Years nine	0.0000	0.0000	0.0000	0.0000
Age ten	Years ten	0.0000	0.0000	0.0000	0.0000
Age elev	Years elev	0.0000	0.0000	0.0000	0.0000
Age twel	Years twel	0.0000	0.0000	0.0000	0.0000
Age thir	Years thir	0.0000	0.0000	0.0000	0.0000
Age four	Years four	0.0000	0.0000	0.0000	0.0000
Age fiv	Years fiv	0.0000	0.0000	0.0000	0.0000
Age six	Years six	0.0000	0.0000	0.0000	0.0000
Age sev	Years sev	0.0000	0.0000	0.0000	0.0000
Age eigh	Years eigh	0.0000	0.0000	0.0000	0.0000
Age nine	Years nine	0.0000	0.0000	0.0000	0.0000
Age ten	Years ten	0.0000	0.0000	0.0000	0.0000
Age elev	Years elev	0.0000	0.0000	0.0000	0.0000
Age twel	Years twel	0.0000	0.0000	0.0000	0.0000
Age thir	Years thir	0.0000	0.0000	0.0000	0.0000
Age four	Years four	0.0000	0.0000	0.0000	0.0000
Age fiv	Years fiv	0.0000	0.0000	0.0000	0.0000
Age six	Years six	0.0000	0.0000	0.0000	0.0000
Age sev	Years sev	0.0000	0.0000	0.0000	0.0000
Age eigh	Years eigh	0.0000	0.0000	0.0000	0.0000
Age nine	Years nine	0.0000	0.0000	0.0000	0.0000
Age ten	Years ten	0.0000	0.0000	0.0000	0.0000
Age elev	Years elev	0.0000	0.0000	0.0000	0.0000
Age twel	Years twel	0.0000	0.0000	0.0000	0.0000
Age thir	Years thir	0.0000	0.0000	0.0000	0.0000
Age four	Years four	0.0000	0.0000	0.0000	0.0000
Age fiv	Years fiv	0.0000	0.0000	0.0000	0.0000
Age six	Years six	0.0000	0.0000	0.0000	0.0000
Age sev	Years sev	0.0000	0.0000	0.0000	0.0000
Age eigh	Years eigh	0.0000	0.0000	0.0000	0.0000
Age nine	Years nine	0.0000	0.0000	0.0000	0.0000
Age ten	Years ten	0.0000	0.0000	0.0000	0.0000
Age elev	Years elev	0.0000	0.0000	0.0000	0.0000
Age twel	Years twel	0.0000	0.0000	0.0000	0.0000
Age thir	Years thir	0.0000	0.0000	0.0000	0.0000
Age four	Years four	0.0000	0.0000	0.0000	0.0000
Age fiv	Years fiv	0.0000	0.0000	0.0000	0.0000
Age six	Years six				

29. The method of claim 27, wherein said reporter gene is *lacZ* or *GFP*.
30. The method of claim 29, wherein said reporter gene is *lacZ*.
- 5 31. The method of claim 29, wherein said reporter gene is *GFP*.
32. The method of claim 31, wherein said reporter gene is a variant of *GFP*.
33. The method of claim 32, wherein said variant is GFPmut2.
- 10 34. The method of claim 27, wherein said mutant strain of *Pseudomonas aeruginosa* comprises a promoterless reporter gene inserted at a genetic locus in the chromosome of said *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID
- 15 NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID
- 20 NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36.
35. The method of claim 34, wherein said promoterless reporter gene is inserted in said chromosome at a locus comprising a nucleotide sequence selected from
- 25 the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:28 and SEQ ID NO:35.
36. The method of claim 34, wherein said reporter gene is contained in a transposable element.
- 30 37. A mutant strain of *Pseudomonas aeruginosa* comprising a promoterless reporter gene inserted at a genetic locus in the chromosome of said *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID
- 35 NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID

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NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36.

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providing a wild type strain of *Pseudomonas aeruginosa* which produces a quorum sensing signal molecule;

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Variable	Mean	SD	Min	Max
Age	34.5	10.2	21	55
Gender				
Male	52.1	49.8	0	100
Female	47.9	50.2	0	100
Marital status				
Married	68.3	48.5	0	100
Single	31.7	49.5	0	100
Education				
High school or less	25.4	43.2	0	100
Some college	32.1	47.1	0	100
Bachelor's degree	42.5	49.8	0	100
Master's degree	18.7	41.5	0	100
PhD	1.1	3.2	0	100
Income				
Less than \$10,000	12.3	34.5	0	100
\$10,000-\$20,000	28.7	45.6	0	100
\$20,000-\$30,000	35.2	48.9	0	100
\$30,000-\$40,000	18.9	40.1	0	100
\$40,000-\$50,000	5.8	23.7	0	100
\$50,000 or more	6.1	24.3	0	100
Health status				
Excellent	15.2	36.8	0	100
Very good	22.5	42.1	0	100
Good	38.7	49.5	0	100
Fair	18.9	40.2	0	100
Poor	5.7	23.4	0	100
Exercise frequency				
Never	10.1	31.2	0	100
Once a week	25.3	44.5	0	100
Two or three times a week	35.6	48.7	0	100
Four or five times a week	18.2	40.3	0	100
Six or seven times a week	10.8	32.1	0	100
Every day	0.1	1.2	0	100
Stress level				
Low	12.5	35.4	0	100
Moderate	28.9	46.7	0	100
High	58.6	49.8	0	100
Life satisfaction				
Very satisfied	18.3	41.2	0	100
Satisfied	32.7	47.5	0	100
Dissatisfied	49.1	50.1	0	100
Very dissatisfied	1.9	4.2	0	100

contacting said mutant strain with said quorum sensing signal molecule and a test compound; and

- 5 detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in *Pseudomonas aeruginosa*.

45. The method of claim 44, wherein said reporter gene is contained in a transposable element.

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46. An isolated nucleic acid molecule comprising a nucleotide sequence, said nucleotide sequence comprising:

15 a regulatory sequence derived from the genome of *Pseudomonas aeruginosa*, wherein said regulatory sequence regulates a quorum sensing controlled genetic locus of the *Pseudomonas aeruginosa* chromosome, and wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; and

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a reporter gene operatively linked to said regulatory sequence.

47. An isolated nucleic acid molecule comprising a quorum sensing controlled genetic locus derived from the genome of *Pseudomonas aeruginosa*, wherein
30 said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID

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51. A vector comprising the isolated nucleic acid molecule of any one of claims 46, 47, 48 and 49.

53. A method for identifying a modulator of quorum sensing signaling in
5 bacteria, said method comprising:

10 contacting said cell with a quorum sensing signal molecule in the presence and
absence of a test compound;

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55. The compound of claim 54, which inhibits quorum sensing signaling in
20 *Pseudomonas aeruginosa* by inhibiting an enzyme involved in the synthesis of a quorum
sensing signal molecule, by interfering with quorum sensing signal reception , or by
scavenging the quorum sensing signal molecule.

56. A method for identifying a quorum sensing controlled gene in bacteria,
25 said method comprising:

providing a cell which is responsive to a quorum sensing signal molecule such that expression of a quorum sensing controlled gene is modulated, and wherein modulation of the expression of said quorum sensing controlled gene generates a detectable signal;

contacting said cell/with a quorum sensing signal molecule;

and detecting a change in the detectable signal to thereby identify a quorum
35 sensing signaling controlled gene in bacteria.

57. The method of claim 56, wherein said cell further comprises means for generating said detectable signal.

Parameter	Estimate	Standard Error	t-Statistic	p-Value
Intercept	0.0000	0.0000	0.0000	0.0000
Age	0.0000	0.0000	0.0000	0.0000
Age squared	0.0000	0.0000	0.0000	0.0000
Age cubed	0.0000	0.0000	0.0000	0.0000
Age quartic	0.0000	0.0000	0.0000	0.0000
Age quintic	0.0000	0.0000	0.0000	0.0000
Age sextic	0.0000	0.0000	0.0000	0.0000
Age septic	0.0000	0.0000	0.0000	0.0000
Age octic	0.0000	0.0000	0.0000	0.0000
Age nonic	0.0000	0.0000	0.0000	0.0000
Age decic	0.0000	0.0000	0.0000	0.0000
Age undecic	0.0000	0.0000	0.0000	0.0000
Age duodecic	0.0000	0.0000	0.0000	0.0000
Age tredecic	0.0000	0.0000	0.0000	0.0000
Age quattuordecic	0.0000	0.0000	0.0000	0.0000
Age quindecic	0.0000	0.0000	0.0000	0.0000
Age sexdecic	0.0000	0.0000	0.0000	0.0000
Age septendecic	0.0000	0.0000	0.0000	0.0000
Age octodecic	0.0000	0.0000	0.0000	0.0000
Age novemdecic	0.0000	0.0000	0.0000	0.0000
Age vigintic	0.0000	0.0000	0.0000	0.0000
Age unvigintic	0.0000	0.0000	0.0000	0.0000
Age bivigintic	0.0000	0.0000	0.0000	0.0000
Age trivigintic	0.0000	0.0000	0.0000	0.0000
Age quadravigintic	0.0000	0.0000	0.0000	0.0000
Age quinquavigintic	0.0000	0.0000	0.0000	0.0000
Age sexavigintic	0.0000	0.0000	0.0000	0.0000
Age septuavigintic	0.0000	0.0000	0.0000	0.0000
Age octuavigintic	0.0000	0.0000	0.0000	0.0000
Age nonuavigintic	0.0000	0.0000	0.0000	0.0000
Age decuavigintic	0.0000	0.0000	0.0000	0.0000
Age undecuavigintic	0.0000	0.0000	0.0000	0.0000
Age duodecuavigintic	0.0000	0.0000	0.0000	0.0000
Age tredecuavigintic	0.0000	0.0000	0.0000	0.0000
Age quattuordecuavigintic	0.0000	0.0000	0.0000	0.0000
Age quindecuavigintic	0.0000	0.0000	0.0000	0.0000
Age sexdecuavigintic	0.0000	0.0000	0.0000	0.0000
Age septendecuavigintic	0.0000	0.0000	0.0000	0.0000
Age octodecuavigintic	0.0000	0.0000	0.0000	0.0000
Age novemdecuavigintic	0.0000	0.0000	0.0000	0.0000
Age viginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age unviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age biviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age triviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age quadraviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age quinquaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age sexaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age septuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age octuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age nonuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age decuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age undecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age duodecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age tredecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age quattuordecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age quindecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age sexdecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age septendecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age octodecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0000
Age novemdecuaviginticuavigintic	0.0000	0.0000	0.0000	0.0

58. The method of claim 57, wherein said signal generation means comprises a reporter gene, and wherein modulation of the expression of said quorum sensing controlled gene modulates transcription of said reporter gene, said transcription providing said detectable signal.

59. The method of claim 58, wherein said reporter gene is operatively linked to a regulatory sequence of said quorum sensing controlled gene.

60. The method of claim 58, wherein said reporter gene is opertively linked to said quorum sensing controlled gene.

61. The method of either of claims 59 and 60, wherein said reporter gene is contained in a transposable element.

62. The method of claim 58, wherein said reporter gene is selected from the group consisting of *ADE1*, *ADE2*, *ADE3*, *ADE4*, *ADE5*, *ADE7*, *ADE8*, *ASP3*, *ARG1*, *ARG3*, *ARG4*, *ARG5*, *ARG6*, *ARG8*, *ARO2*, *ARO7*, *BARI*, *CAT*, *CHO1*, *CYS3*, *GAL1*, *GAL7*, *GAL10*, *GFP*, *HIS1*, *HIS3*, *HIS4*, *HIS5*, *HOM3*, *HOM6*, *ILV1*, *ILV2*, *ILV5*, *INO1*, *INO2*, *INO4*, *lacZ*, *LEU1*, *LEU2*, *LEU4*, *luciferase*, *LYS2*, *MAL*, *MEL*, *MET2*, *MET3*, *MET4*, *MET8*, *MET9*, *MET14*, *MET16*, *MET19*, *OLE1*, *PHO5*, *PRO1*, *PRO3*, *THR1*, *THR4*, *TRP1*, *TRP2*, *TRP3*, *TRP4*, *TRP5*, *URA1*, *URA2*, *URA3*, *URA4*, *URA5* and *URA10*.

63. The method of claim 56, wherein said quorum sensing signal molecule is produced by a second cell.

64. The method of claim 63, wherein said second cell is a prokaryote or eukaryote.

65. The method of claim 64, wherein said second cell is a bacterium.

66. The method of claim 56, wherein said cell is a prokaryote or eukaryote.

67. The method of claim 66, wherein said cell is a bacterium.

68. The method of either of claims 65 and 67, wherein said bacterium is a gram negative bacterium.

74. The method of claim 56, wherein said quorum sensing signal molecule induces the expression of said quorum sensing controlled gene.

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